

Amendments to the Specification:

After page 24 of the specification, please insert the paper copy of the Sequence Listing enclosed herewith (2 pages).

Please delete the paragraph on page 21, line 3 to page 22, line 18 and replace it with the following paragraph:

GGTA1 heterozygous 355-F1 cells were isolated from a nuclear transfer fetus (355-F1) at day 32 of gestation and cultured as described (Lai et al., Science 295:1089. The donor cells for reconstruction of nuclear transfer embryos leading to the fetuses were explanted and cultured from ear sections of pig O212-2, a GGTA1 heterozygote in which one allele has been inactivated by homologous recombination with vector pGalGTAS-Neo (Lai et al., Science 295:1089, 2002). Cells were cultured in F10 medium containing 20% FBS and 20 µg/ml gentamycin (media) on collagen I coated dishes at 5%CO₂, 3%O₂, and 37°C. The cells were then treated with antibody and complement twice in suspension as above prior to plating at low density for isolation of clones lacking α(1,3)-gal epitope expression. After the second treatment, cells were plated at 5 and 10 cells/well in collagen I coated 96well plates. In situ treatments with 100-500 µg/ml anti-α(1,3)-gal antibody for 1 hr 37°C and 1:8 rabbit complement in for 1 hr 37°C were performed every other day for treatments 3-5. Wells containing patches of cells covering greater than 15% of the well were transferred to a 48 well plate and treated the following day in situ with 500 µg/ml anti-α1,3-gal antibody and complement. Cells were passaged for molecular analysis, IB4-FITC analysis, and freezing. RNA and ethanol precipitated DNA were prepared using RNeasy and DNeasy systems from Qiagen. Wells containing viable cells following the last treatment were cultured without further selection and lysis resistant clones analyzed for epitope and RNA expression, as well as GGTA1 locus structure. Analysis of two representative clones, Q2 and Q9, is shown in Figures 2 and 3. Both clones had little or no specific IB4 binding (Figure 2). Approximately 50 ng of Q2 and Q9 RNA was reversed transcribed into cDNA using AMV Reverse Transcriptase XL (Takara Shuzo Co., Ltd.) cDNA was then amplified in reactions using LA Taq DNA polymerase (Takara Shuzo Co., Ltd.), the GGTA1 exon 2 forward primer GT-598 (5'-TTCTGCAGAGCAGAGCTCAC; **SEQ ID NO: 1**) and the exon 9 reverse primer RN1 (5'-

CCCTCAACCCAGAACAGATAAG; **SEQ ID NO: 2**). PCR products were analyzed on a 1% gel. Southern blots of the RT-PCR products were hybridized to oligonucleotide R823 (5'-AGGATGTGCCTTGTACCACC; **SEQ ID NO: 3**), which detects transcripts derived from both gene targeted and native GGTA1 loci. A 1421 base pair band is expected for the native locus and 2472 base pair band for the targeted locus. RT-PCR analysis of the clones (Figure 3) resulted in a band compatible with expression from the gene targeted locus present in Q2 and Q9 cells, but no band compatible with expression from a wild-type GGTA1 locus. Approximately 200 ng of DNA was amplified, cut and analyzed on agarose gels as described (Lai et al., Science 295:1089), except that the 5' genomic assay utilized primers F248 (5'-GAAGAAGACGCTATAGGCAACG; **SEQ ID NO: 4**) and RN1 in place of F238 and R823. To increase sensitivity of detection, DNA from the gels was transferred to nylon membranes and hybridized to oligonucleotide probe R823 as above. Hybridizing Eco RI bands of approximately 2550 bp and 3600 bp are expected in the 5' genomic assay from native and targeted GGTA1 loci respectively. Hybridizing Sac I bands of approximately 1250 bp and 2300 bp are expected in the 3' genomic assay from native and targeted GGTA1 loci respectively. Genomic PCR analysis (Figure 3), revealed the presence of a GGTA1 locus with a structure expected from the gene targeted locus in 355-F1 cells, but no locus with a structure like that of the wild-type GGTA1 locus.